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A Retrospective Analysis of Twelve Developmental Neurotoxicity Studies Submitted to the USEPA Office of Prevention, Pesticides, and Toxic Substances (OPPTS)

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INTRODUCTION

Since the Food Quality Protection Act (FQPA) was passed in 1996, the completeness and adequacy of the toxicity database currently available for the evaluation of adverse effects of pesticides and toxic substances to infants and children has come under greater scrutiny. In particular, much attention has been focused on the assessment of nervous system development. Guideline studies that evaluate the effects of a chemical on pre- or postnatal structural and physical development have been used for years as indicators of the potential for a substance to affect development of the nervous system. These guideline studies include the prenatal developmental toxicity study and the two-generation reproduction study. In addition, endpoints evaluated in any of the studies conducted in adult animals may lead to concerns regarding nervous system development in the offspring. These studies in adult animals and their offspring have been required for the assessment of all food-use pesticides (40 CFR Part 158.340) and have been selectively required for toxic substances (under the Toxic Substances Control Act). A developmental neurotoxicity (DNT) guideline (which more specifically addresses various indices of neurological development, including brain weight, morphometry, and numerous neurobehavioral measures of offspring following pre- and limited postnatal exposure) has been used by the Office of Prevention, Pesticides, and Toxic Substances (OPPTS) for a select number of chemicals, but is not part of the Office of Pesticide Program's (OPP) current data requirements (40 CFR 158.340).

There have been specific requests from FIFRA Scientific Advisory Panels (1996 and 1998), as well as the Tolerance Reassessment Advisory Committee (TRAC), for an analysis of developmental neurotoxicity study data received to date by OPPTS. Despite the small number of studies available, it was believed, given the broad interest, that these studies and reviews should be brought forward by OPPTS at this time.

This paper provides an initial analysis of twelve developmental neurotoxicity studies evaluated by OPPTS in support of the registration and/or use of pesticides and toxic substances. The results of the studies are summarized, and compared to related studies that evaluate prenatal development, reproduction and fertility, and adult neurotoxicity (acute and subchronic) in the same species (rat). In addition, for the pesticides examined, the placement of these findings within the framework of dietary risk assessment is discussed.

Objectives of this review include ascertaining the potential contribution of the developmental neurotoxicity data to: 1) establishing confidence that potential hazards to neurological development are identified, and 2) estimating risk (acute and chronic RfDs). Such determination must be considered preliminary given the limited number of studies and the lack of breadth of chemical classes evaluated. In the course of this review, various general issues are raised that are relevant to the developmental neurotoxicity study methodology and interpretation of results. They include: the route of administration, the duration of treatment, the use of combined protocols, biochemical measures including cholinesterase inhibition, pharmacokinetic data, and age-related susceptibility. These issues provide the context for the some of the questions that OPPTS will bring before the Scientific Advisory Panel in December, 1998. In addition, the Panel will be asked to comment on a proposed use of developmental neurotoxicity data for the selection of endpoints for risk assessment of pesticides.

A brief history of the developmental neurotoxicity (DNT) testing guideline

In 1988, a developmental neurotoxicity protocol was developed by the Office of Toxic Substances for the assessment of specific solvent chemicals (CFR 795.250). This testing protocol received extensive scientific review. Several of the test procedures had been validated in earlier studies (e.g., Buelke-Sam et al., 1985). In addition, an Agency-wide Workshop on the Qualitative and Quantitative Comparability of Human and Animal Developmental Neurotoxicity was held on April 11-13, 1989 (Kimmel, Rees, and Francis, 1990); the ability of the test guideline to detect agents that were known to cause developmental neurotoxicity in humans was evaluated. The conclusion of the workshop participants was that the guideline would detect neurotoxic effects of each of the agents evaluated, although the outcomes might be different from those seen in humans. The guideline was further considered by the FIFRA Scientific Advisory Panel (SAP) in 1989. Based upon the results of these efforts, an OPPTS developmental neurotoxicity testing guideline (§83-6) was finalized in 1991. It was slightly revised in August, 1998 (OPPTS 870.6300), as part of a larger effort to harmonize testing guidelines with the Organisation for Economic Co-Operation and Development (OECD).

Inclusion of the developmental neurotoxicity testing guideline in 40 CFR Part 158 toxicology testing requirements for pesticides was discussed in a pre-proposal presented to the SAP in 1994. OPPTS has used this guideline as a second tier toxicology test, triggered by findings in the chemical database or other issues of concern, as reviewed by an 1987 SAP (Appendix A-1).

Description of the data set used in the retrospective analysis

A total of nine studies have been submitted to the Office of Pesticide Programs to support the registration or reregistration of specific pesticides, and three studies have been submitted to the Office of Pollution Prevention and Toxics (Table 1). The substances tested represent several chemical classes, as indicated.

Table 1. Chemicals for which a developmental neurotoxicity study has been reviewed by OPPTS

Pesticides		Toxic Substances	
Chemical	Chemical Class	Chemical	Chemical Class
Aldicarb	Carbamate	1,1,1-trichloroethane (1,1,1-TCE)	Chloroalkyl
Carbaryl	Carbamate	Triethylene glycol monomethyl ether (TGME)	Glycol ether
Carbofuran	Carbamate	Isopropanol	Alcohol
Molinate	Thiocarbamate		
N,N-diethyl-m-toluamide (DEET)	Toluamide		
Emamectin	Antibiotic derivative		
Fipronil	Phenyl pyrazole		

Table 1. Chemicals for which a developmental neurotoxicity study has been reviewed by OPPTS			
Pesticides		Toxic Substances	
Chemical	Chemical Class	Chemical	Chemical Class
Chlorpyrifos	Organophosphate		
CHEMICAL X a	Neoalkanamide		
a = A new chemical pesticide, not yet registered.			

The submission of these data was generally related to specific requests from the Agency, following a review of the chemical toxicity profile and, for pesticides, an evaluation of the weight of the evidence that would suggest the need for the developmental neurotoxicity study. For the pesticides tested, exceptions included CHEMICAL X, a non-food use pesticide for which a DNT study was submitted voluntarily by the Registrant, and DEET for which extensive testing was submitted by the Registrant to evaluate all aspects of the toxicological profile. For each of these chemicals, weight-of-evidence considerations that identify a concern and support a recommendation that a developmental neurotoxicity study be conducted are summarized in Table 2.

Table 2. Weight of evidence considerations that support a recommendation for DNT testing

Chemical	Neuropath-ology	Endocrine disruption	Behavioral/ Functional Effects	SAR	Neurotoxic Potency a	Other Information or Concerns
Aldicarb			X	X	X	
Carbaryl			X	X	X	Effects on development and reproduction in various species
Carbofuran			X	X	X	
Molinate	X	X		X		Interferes with testosterone biosynthesis
DEET			X			Widespread potential for exposure to children
Emamectin	X		X			Neuropathology and neuro-behavioral findings in pups on 2-generation reproduction study; ion channel activation
Fipronil		X	X			GABA disrupter (ion channel blocker)

Table 2. Weight of evidence considerations that support a recommendation for DNT testing

Chemical	Neuropath-ology	Endocrine disruption	Behavioral/ Functional Effects	SAR	Neurotoxic Potency a	Other Information or Concerns
Chlorpyrifos	X		X	X	X	Literature studies identified other concerns re: neurological development and susceptibility to offspring Widespread potential for exposure to children
CHEMICAL X						No findings of concern
1,1,1-TCE			X	X		ITC recommendation for testing based on existing studies; widespread potential for exposure
TGME			X	X		b
Isopropanol			X	X		b

a = "Neurotoxic potency" is based on observations of neurotoxic effects at low doses, but may also include issues such as the persistence and/or partitioning of effects.
b = See comment for 1,1,1-TCE
ITC = Interagency Testing Committee

This paper does not include an evaluation of the scientific rationale, or the criteria or weight-of-evidence considerations, that have been used in the past, are currently in use, or may be used in the future to determine the need for a developmental neurotoxicity study. Information on those criteria is provided in Appendix A-1, solely for the purpose of placing the weight-of-evidence summarized in Table 2 into a broader perspective. In addition, Appendix A-2 contains a list of pesticides for which an OPP peer review committee (RfD or HIARC) has recommended a developmental neurotoxicity study be conducted (but which has not been received or reviewed at this time) and a checklist summary of the findings that resulted in those recommendations. This list is also provided only as supplemental information to the retrospective analysis of the twelve OPPTS developmental neurotoxicity studies that are listed in Table 1.

The developmental neurotoxicity study protocol

In a study conducted according to the standard developmental neurotoxicity study guideline (OPPTS 870.6300), pregnant rats are administered the chemical orally from gestation day 6 through postnatal day 10. These testing days are defined in relation to the day of mating and the day of birth, designated as gestation and postnatal (lactation) days 0, respectively. The offspring are therefore exposed to the chemical, via the maternal circulation and/or milk, during *in utero* and early postnatal development for approximately 25 days.

The dams are examined grossly at least once daily before treatment, and detailed clinical observations are conducted (outside of the home cage) on approximately half of the dams in each group twice during gestation and twice during lactation. Maternal body weight is recorded at least weekly.

The offspring are assessed for evidence of deficits in neurobehavioral development. Litters are randomly standardized on postnatal day 4 to yield four pups per sex per litter, and the pups are assigned for testing. Endpoints evaluated between birth and day 60 of age include measures of physical development (including sexual maturation), motor activity, auditory startle reflex function, and learning and memory. Daily cage-side observations are conducted, and 10 pups/sex/group receive detailed clinical observations outside the cage on days 4, 11, 21, 35, 45, and 60. Pups are counted and weighed individually at birth; on postnatal days 4, 11, 17, 21; and at least once every 2 weeks thereafter. The age of vaginal opening and preputial separation are recorded. Motor activity is monitored by an automated activity recording apparatus on postnatal days 13, 17, 21, and 60 ($\pm$2). Tests of auditory startle habituation and associative learning and memory are performed on the offspring around the time of weaning (day 21) and around day 60. Flexibility is allowed in the choice of tests for learning and memory, although the guideline provides criteria for selection and some examples of tests that could be used.

At postnatal day 11 and at study termination, the offspring are subjected to extensive neuropathological examination including simple morphometric analysis. One pup per sex per litter is killed on day 11. Of these, six pups per sex per group are assigned to neuropathological evaluation; their brains are removed and immersed in an aldehyde fixative. At study termination, all remaining offspring are killed; six of these rats per sex per group are prepared for neuropathological evaluation with *in situ* transcardial perfusion of appropriate fixatives (paraformaldehyde and gluteraldehyde). Brain weight is recorded at both a preweaning timepoint (postnatal day 11) and at study termination (postnatal day 60). Qualitative neuropathological examination is conducted for the control and high-dose groups, and if a treatment-related finding is evident, the mid- and low-dose groups are successively examined. Guidance is provided regarding the regions of the brain to be examined and the types of alterations upon which to focus, particularly emphasizing structural changes indicative of developmental insult. Simple morphometric analysis, performed on offspring killed at postnatal day 11 and at termination, is defined as consisting, at a minimum, of a reliable estimate of the thickness of major layers at representative locations within the neo-cortex, hippocampus, and cerebellum.

<u>The DNT study methods for the twelve chemicals analyzed</u>

The protocols for the developmental neurotoxicity studies that were submitted to the Agency are summarized briefly in the tables and text below. TGME and isopropanol were conducted using the developmental neurotoxicity screening protocol (CFR 795.250). The testing guidelines for 1,1,1-TCE and DEET were developed external to the Agency. All of the other pesticides were conducted using the OPPTS developmental neurotoxicity study testing guideline (§83-6).

• Methods: maternal animals

In each of the studies, the maternal animals were treated, at a minimum, from the time of implantation, through delivery of the litter, and until day 10 of the lactation period (Table 3A).

Table 3A. Study protocols: maternal treatment

Chemical	Dose Levels (mg/kg/day) a	Route of Administration	Duration b
Aldicarb	0.05, 0.10, 0.30	Gavage	GD 6 thru PND 10
Carbaryl	0.1, 1.0, 10	Gavage	GD 6 thru PND 10
Carbofuran	1.7, 6.9, 31 (20, 75, 300 ppm)	Diet	GD 6 thru PND 10
Molinate	1.8, 6.9, 26.1 (20, 75, 300 ppm)	Diet	GD 6 thru PND 10
DEET	22.5, 90, 225 (500, 2000, 5000 ppm)	Diet	GD 0 thru PND 330 (11 months) c
Emamectin	0.1, 0.6, 3.6/2.5 d	Gavage	GD 6 thru PND 20
Fipronil	0.05, 0.9, 18.5 (0.5, 10, 200 ppm)	Diet	GD 6 thru PND 10
Chlorpyrifos	0.3, 1, 5	Gavage	GD 6 thru PND 11
CHEMICAL X	40, 125, 400	Gavage	GD 6 thru PND 11e
1,1,1-TCE	75, 250, 750	Gavage	GD 6 thru PND 10
TGME	300, 1650, 3000	Gavage	GD 6 thru PND 21
Isopropanol	200, 700, 1200	Gavage	GD 6 thru PND 21

a = For dietary studies: dose levels for carbofuran and fipronil represent the highest value calculated for dams in the test group; dose levels for molinate represent the value for dams during gestation only; dose levels for DEET are representative of P males.
b = PND 10 and PND 11 are equivalent, based upon the designation of the day of litter birth as either PND 0 or PND 1.
c = Conducted as a segment of a 2-generation reproduction study. F2 offspring of treated F1 dams were maintained on treated diet for 9 months postweaning and then evaluated for functional endpoints over the following 8 weeks (40-48 weeks of age).
d = The high dose was reduced to 2.5 mg/kg/day on GD 17-20 due to tremors observed in pups at 3.6 mg/kg/day on a concurrently conducted reproduction study.
e = Conducted as a segment of a combined prenatal developmental/developmental neurotoxicity study.

The route of administration to the dams was either by diet or gavage in all of the studies reviewed. In three of the studies (emamectin, TGME, and isopropanol) treatment of the dams was extended to the end of the lactation period, at which time the pups were weaned, and in one study (DEET) the test substance was administered continuously in the diet to the dams and offspring until termination. Administration of the test substance in the diet continuously throughout lactation allows for direct consumption of the material by the offspring during the late lactation period (approximately postnatal days 15-21). In the twelve studies reviewed for this retrospective analysis, it was noted that the test substance was not administered directly to the offspring in any study with the exception of the one conducted with DEET; all other dietary studies terminated exposure at day 10 or 11.

For two of the pesticides evaluated, the developmental neurotoxicity studies were conducted as combined study protocols, either as a segment of a two-generation reproduction study (DEET) or in conjunction with a prenatal developmental toxicity study (CHEMICAL X).

Maternal observations for all studies included, at a minimum, clinical observations, body weight and food consumption data, and a cursory gross pathology examination. More detailed maternal endpoints, e.g., motor activity testing on dams at the time of peak effect of the test material, or the evaluation of cholinesterase activity, were seldom evaluated. Although this did not affect the adequacy of any of the studies examined, it did have other implications, such as in the interpretation of differential effects of treatment on the dams versus the offspring.

Several of the chemicals analyzed in this paper were cholinesterase inhibitors. The guideline for developmental neurotoxicity testing does not specify that data on cholinesterase inhibition (or any other neurochemical biomarker) be evaluated. However, maternal blood and brain cholinesterase activity data were collected in three of the pesticide studies (aldicarb, carbaryl, and chlorpyrifos), from subsets of animals assigned specifically for that purpose. For aldicarb, maternal plasma and erythrocyte cholinesterase measurements were recorded prior to dosing and at gestation day (GD) 7 and lactation days (LD) 7 and 11. For carbaryl, maternal plasma, erythrocyte, and whole blood cholinesterase measurements were recorded prior to dosing, at GD 6, 15, and 20, and at LD 4 and 10. For chlorpyrifos, plasma and erythrocyte cholinesterase measurements were performed at GD 20. Maternal brain cholinesterase determinations were performed at LD 11 for aldicarb, at LD 10 for carbaryl, and at GD 20 for chlorpyrifos. Cholinesterase inhibition was not examined in the developmental neurotoxicity studies on carbofuran and molinate, both of which have been shown (in other studies in the database) to inhibit cholinesterase activity, although it is noted that this effect has been shown to occur only at a marginal response level at high doses for molinate. For fipronil and emamectin, the general mechanism of neurotoxicity is known, but no biomarker was apparent or measured. For the other chemicals tested (DEET, CHEMICAL X, 1,1,1-TCE, TGME, and isopropanol) neither the mechanism of action nor any neurochemical biomarker of exposure or toxicity was assessed.

• Methods: offspring

Observations on the offspring for the twelve studies are characterized in Tables 3B-1 through 3B-3. All studies contained multiple assessments of offspring physical development and maturation, behavioral and/or functional observations, motor activity, auditory startle habituation, learning and memory, brain weight, and neuropathology. For some endpoints, the methodologies and days of testing were consistent across the chemicals evaluated, e.g., motor activity assessments or body weight measurements as an indicator of physical development. For others, such as testing of learning and memory, the guideline allows more flexibility in study design, resulting in a much greater variability in the tests selected, the equipment and methodologies used, and the information obtained.

Neurochemical biomarker measurements in offspring are not included in the generic developmental neurotoxicity study guideline. However, on the aldicarb study, blood and brain cholinesterase activity were measured in pups at postnatal days 4, 10, and 11. Neither fetal nor postnatal cholinesterase measurements were collected in any other study examined in this retrospective analysis; however, these data were examined in a companion study for chlorpyrifos. In that study, maternal rats were administered the test chemical using the same treatment regime as in the developmental neurotoxicity study (Mattsson et al., 1998). Briefly, blood and milk samples were collected from dams and blood samples were collected from offspring, for the determination of levels of chlorpyrifos and two metabolites (chlorpyrifos-oxon and 3,5,6-trichloro-2-pyridinol [TCP]), on gestation day 20 and lactation days 1, 5, and 11. Cholinesterase activity was measured in blood (plasma and RBC) and tissue (brain and heart) samples taken from dams and offspring on gestation day 20 and lactation days 1, 5, 11, 22, and 65 (offspring only). Measurable levels of chlorpyrifos and/or its metabolites were demonstrated in dam and offspring blood during gestation and/or lactation for all treated groups in a dose-related pattern. Notably, milk concentrations of chlorpyrifos on lactation day 1 and 5 were at least 10-fold greater than blood concentrations in all dose groups. Cholinesterase was inhibited in dams of all treated groups with approximately the same profile as in the developmental neurotoxicity study (plasma ChEI at the low-dose, RBC and brain ChEI at the mid- and high-doses); however, inhibition of cholinesterase activity in fetuses and pups was only observed at the high-dose. A similar pattern of greater brain cholinesterase inhibition in dams as compared to fetuses was also noted by Lassiter et al. (1998); this was interpreted as a greater ability of fetal brain ChE to recover between each dose, as compared to maternal brain ChE. These supplementary data provide valuable information that support and more clearly define the adequacy of dosing and the resultant findings of the developmental neurotoxicity study with chlorpyrifos.

Table 3B-1. Study protocols: Offspring physical development

Chemical	Body Weight Measurements (postnatal day)	Physical Maturation (day attained) a			
		Eye opening	Incisor eruption	Pinna detachment	Sexual maturation
Aldicarb	0, 7, 11, 17, 21, biweekly				X
Carbaryl	0, 4, 7, 11, 13, 17, 21	X	X		X
Carbofuran	0, 4, 11, 17, 21	X	X	X	X
Molinate	1, 5, 12, 18, 22, 29, weekly				X
DEET	Weekly starting at Wk 40				
Emamectin	0, 4, 11, 17, 21				X
Fipronil	0, 4, 11, 17, 21, weekly	X	X	X	X
Chlorpyrifos	1, 5, 12, 18, 22, 40, 66	X		X	X
CHEMICAL X	1, 5, 8, 12, 14, 18, 22, weekly				X
1,1,1-TCE	1, 4, 7, 13, 17, 21, 28	X	X	X	X
TGME	1, 4, 7, 13, 17, 21, 35, 49, 68				X
Isopropanol	0, 4, 7, 13, 17, 21, 36, 49, 68				X

a = X designates that the parameter was examined.

Table 3B-2. Study protocols: Offspring neurobehavioral testing a

Chemical	FOB	Motor Activity	Auditory Startle c	Learning and Memory	Other testing
Aldicarb	14, 21, 38, 63	13, 17, 21, 60	22, 60	Water M-maze: 23, 24, 25, 30, 60	
Carbaryl	4, 7, 11, 13, 17, 21	13, 17, 21, 60	22, 60	Water E-maze: 60-65 Passive avoidance: 23	Swimming abnormality
Carbofuran		13, 17, 22, 60	22, 60	Water Y-maze: 24, 60	Swimming development (angle, direction, paddling): 6, 8, 10, 12, 14
Molinate	b	14, 18, 22, 60	23, 61	Water Y-maze: 21, 59	Swimming ability in straight channel: 24, 62
DEET	X	Wks 41-42	Wk 46, 47	Water M-maze: Wk 43-45 Passive avoidance: Wk 48	Grip strength: Wk 41 Thermal response: Wk 41
Emamectin	b	13, 17, 21, 59	22, 59	Passive avoidance: 24, 31, 59, 66	
Fipronil		13, 17, 22, 60	22, 60	Water Y-maze: 24, 60	Swimming development (angle, direction, paddling): 6, 8, 10, 12, 14
Chlorpyrifos	b 40, 66	14, 18, 22, 61	23, 62	T-maze (spatial delayed alternation): 23-25, 62-92	
CHEMICAL X	b	14, 18, 22, 61	23, 61	Water M-maze: 23/24, 30/31 Passive avoidance: 24	
1,1,1-TCE	45, 60	13, 17, 21, 44, 59	22, 61 d	Delayed matching to position: 65	Grip strength: 45, 60
TGME		13, 17, 21, 47, 58	22, 60	Active avoidance: 60-64	
Isopropanol		13, 17, 21, 47, 58	22, 60	Active avoidance: 60-64	

a = X designates that the test was conducted; numbers represent postnatal day of testing.
b = Detailed clinical observations.
c = With habituation.
d = Described as “auditory brainstem response”: composite waveforms, qualitative and quantitative assessments.

Table 3B-3. Study protocols: Offspring neuropathological testing a					
Chemical	Day of Tissue Collection	Brain Weight b	Brain Gross Measurements	Microscopic Neuropathology	Morphometric Analysis
Aldicarb	11, 60	X	X	X	X c
Carbaryl	11, 70	X		X	X
Carbofuran	11, 60	X		X	
Molinate	12, 63	X	X	X	X
DEET	Wk 48			X	
Emamectin	11, 60	X		X	X
Fipronil	11, 60	X		X	d
Chlorpyrifos	12, 66-71	X		X	X
CHEMICAL X	12, 74-93	X		X	X
1,1,1-TCE	28, 62	X	X	X	
TGME	22, 68	X		X	
Isopropanol	22, 68	X		X	

a = X designates that the test was conducted; numbers represent postnatal day of testing.
b = Brain weight generally determined after fixation.
c = Offspring evaluated on PND 60, but not PND 11.
d = Qualitative evaluation only.

As indicated in Table 3B-3, for the nine pesticide studies evaluated, brain neuropathological evaluation included simple morphometric analysis in all but three of the studies (carbofuran, DEET, and fipronil). In addition, in the carbaryl study, morphometric analysis was conducted for control and high-dose groups only, and examination of the low- and mid-dose groups was not performed, although effects were observed at the high-dose. For aldicarb, morphometric analysis was only conducted on postnatal day 60, but not postnatal day 11. Morphometry was not evaluated for any of the three solvents examined.

The DNT study results

The results of the developmental neurotoxicity studies are summarized in Tables 4A (maternal findings) and 4B (offspring findings). For these studies, no-observed-effect levels (NOELs) and lowest-observed-effect levels (LOELs) are presented in the tables, rather than NOAELs and LOAELs, since the Data Evaluation Reports for most of the studies present the values in this manner. The Agency's consensus risk assessment practices have been designed to identify and characterize adverse effects, in other words, those that would be of regulatory concern. The appropriate acronyms to be used in describing such adverse effects, which are identified in most studies, would be the NOAEL and LOAEL. Generally, the NOELs identified by HED were actually NOAELs for the effects of concern.

• Results: maternal

For most of the chemicals tested, the findings in maternal animals, which primarily consisted of decreased body weight and food consumption and/or clinical signs indicative of neurotoxicity, demonstrated adequate dose selection (Table 4A). For emamectin and 1,1,1-TCE, no maternal toxicity was demonstrated; however, for emamectin, neurobehavioral and neuropathological findings in the offspring indicated that the dose levels were adequate (Table 4B). For 1,1,1-TCE no offspring effects were noted, suggesting that the high dose level may not have been sufficient.

• Results: offspring

As shown in Table 4B, observations that were indicative of a treatment-related effect on nervous system development were noted in seven of the nine pesticides examined (aldicarb, carbaryl, carbofuran, molinate, emamectin, fipronil, and chlorpyrifos). These findings included delays in physical development; alterations in function (e.g., grip strength, hindlimb splay, swimming ability), motor activity, startle reflex, learning, or memory; decreases in brain weight; and/or decreases in morphometric measurements. In the other two pesticides, DEET and CHEMICAL X, findings in the offspring were limited to changes in motor activity at the highest doses tested (in addition to reduced pup weight for CHEMICAL X). For the solvent, TGME, alterations of the startle response (amplitude and latency) at the highest dose tested were observed. In these three studies, the data provide only limited evidence of a specific treatment-related neurotoxic effect. The Guideline for Neurotoxicity Risk Assessment (1998), recommends that "*although interpretation of developmental neurotoxicity may be limited, it is clear that functional effects should be evaluated in light of other toxicity data, including other forms of developmental toxicity....The level of confidence that an agent produces an adverse effect may be as important as the type of change seen, and confidence may be increased by such factors as reproducibility of the effect, either in another study of the same function or by convergence of data from tests that purport to measure similar functions. A dose-response relationship is an extremely important measure of a chemical's effect; in the case of developmental neurotoxicity, both monotonic and biphasic dose-response curves are likely, depending on the function being tested.*" For the other two solvents that were examined (1,1,1-TCE and isopropanol), there were no findings in the offspring.

When developmental neurotoxicity is observed in the presence of maternal toxicity, it is often difficult to determine if the findings in young pups are secondary to the maternal toxicity. For example, decreased pup survival during early lactation and/or perinatal alterations in behavioral findings may be related to other events in either the offspring or the dam, such as an increase in the amount of the test substance in the milk at a critical time of development, inability of the offspring to suckle, general toxicity to the offspring, or insufficient maternal care of the litter. In the twelve studies reviewed in this paper, the interpretation of such findings was conducted in accordance with the Agency Guidelines for Developmental Toxicity Risk Assessment (1991) which states that "*when adverse developmental effects are produced only at doses that cause minimal maternal toxicity, ...the developmental effects are still considered to represent developmental toxicity and should not be discounted as secondary to maternal toxicity.*"

Significant brain weight decreases were noted in five of the nine pesticide studies examined, on PND 11 only (carbofuran and chlorpyrifos), both PND 11 and 60 (molinate and fipronil), or PND 60 only (emamectin). Consistent with the Agency Guideline for Neurotoxicity Risk Assessment (1998), the significant brain weight decrements were judged to represent adverse treatment-related effects. The guideline states that "*A change in brain weight is considered to be a biologically significant effect. This is true regardless of changes in body weight, because brain weight is generally protected during undernutrition or weight loss, unlike many other organs or tissues. It is inappropriate to express brain weight changes as a ratio of body weight and thereby dismiss changes in absolute brain weight*." The regulation of brain weight during development is robust and tends to be maintained even when insults such as malnutrition result in a marked reduction in body weight (Dobbing, 1970; Allen, 1995). Consequently, absolute brain weight is an appropriate indicator of impaired neurologic development, even though the impairment may be mediated by such influences as litter size, body weight at birth, or hormonal or placental insufficiency. The detection of reduced brain weight on PND 11 but not PND 60 may be indicative of alterations in pre- or early postnatal development, resulting in persistent morphological effects (e.g., reduction in the number of neurons) that are masked by subsequent addition of a large extent of brain mass associated with processes such as cell growth and myelination. Reductions detected at both PND 11 and 60 may be related to substantial prenatal effects that have cascading effects on subsequent development, or alternatively, more moderate impairments occurring during both pre- and postnatal brain development. The most parsimonious interpretation of a reduction in brain weight on PND 60, but not PND 11, is the disruption of later postnatal events, such as the acquisition of granule cells in the cerebellum and hippocampus or the myelination of major tracts within the brain. In spite of the fact that alteration in brain weight may not be a sensitive indicator of impaired neurological development, the findings of these studies are consistent with its use as an indicator of impaired brain development in the context of developmental neurotoxicity testing.

Treatment-related alterations in brain morphology were revealed by morphometric analysis for three of the six pesticides for which these data were available (carbaryl, molinate, and chlorpyrifos). While morphometric alterations correlated with decreases in brain weight, two aspects of these results are consistent with morphometric analysis being a more important indicator of altered brain morphogenesis than brain weight. First, in the case of carbaryl, morphometric analysis detected alteration at doses where no changes in brain weight or body weight were apparent. Second, in the case of chlorpyrifos, morphometric alterations that were detectable at PND 11 were still detectable at PND 62, when significant differences in brain weight were not detected. The apparent greater sensitivity of morphometric analysis compared to brain weight may be due to two considerations: 1) alteration in specific and localized brain regions that are detected with morphometric analysis may not entail sufficient changes in mass to exceed normal variance in brain weight, and 2) flexibility in the guidelines that permit the neuropathologist to use professional judgement to select the most appropriate regions to measure and the most appropriate method of measurement. It is additionally noted that the changes in morphometric parameters were generally consistent with functional changes, supporting the biological plausibility that the test compound had an effect on neural development in the offspring.

<u>Placement of the DNT study findings into the overall developmental, reproductive, and neurotoxicity database for rats</u>

For the chemicals under review, the results of the rodent prenatal developmental toxicity study, the multigeneration reproduction study, and the acute and subchronic neurotoxicity studies are summarized in Tables 5 through 8, respectively. While it is recognized that the developmental neurotoxicity study for any chemical should be considered and interpreted in the context of the entire toxicity database, these specific studies were selected for comparison in this paper because they include assessments of toxicity to perinatal animals or because they include neurobehavioral or neuropathological endpoints. In general, these studies were conducted according to standard EPA testing guidelines and were judged to be acceptable.

Table 9 provides a list of the studies and endpoints that were selected by HED peer review committees for the acute and chronic dietary risk assessments of the pesticides discussed. The information in this table is included for comparative purposes only. (It is noted that at this time, OPP has proposed to derive the RfDs for three chemicals, aldicarb, carbofuran, and chlorpyrifos, using human study data. These decisions may be revised in the future, pending the outcome of the December, 1998 SAP/SAB meeting on human testing.) The developmental neurotoxicity study endpoints and associated NOELs were selected for acute dietary risk assessment for two chemicals. For carbaryl, effects from maternal FOB findings that were observed following a single dose (e.g., on GD 7) were used. For molinate, reduced auditory startle in the offspring was used, based upon the assumption that developmental neurotoxicity could have resulted from a single exposure to the chemical. A developmental neurotoxicity study maternal clinical observation endpoint was also used for a short-term (1-7 days) dietary assessment for incidental hand-to-mouth oral residential exposure to infants and children for CHEMICAL X, a chemical with no anticipated food uses. The sensitivity of the maternal endpoints may be a result of the increased sensitivity of the pregnant dams or other non-specific factors like dose selection.

The developmental neurotoxicity study did not provide an endpoint for chronic dietary risk assessment for any of the nine chemicals discussed, although it is noted that in one instance (carbaryl) the NOEL for the chronic dietary endpoint selected from a chronic canine study (1.4 mg/kg/day, based on plasma and brain cholinesterase inhibition) is slightly greater than either the maternal or offspring NOEL from the developmental neurotoxicity study (1.0 mg/kg/day, based on findings including cholinesterase inhibition and cholinergic signs in dams and on decreased hindlimb grip strength and splay and decreased motor activity in offspring) (also see Tables 4A and 4B). A decision was made to utilize the chronic study endpoint in risk assessment, since the study is more representative of a lifetime dietary exposure scenario, and because the dose values of 1.0 and 1.4 mg/kg/day are considered essentially equivalent for risk assessment calculations.

In Table 10, the previously detailed NOELs from the developmental neurotoxicity, prenatal developmental toxicity, multigeneration reproduction, and neurotoxicity studies are presented side-by-side with the NOELs selected for acute and chronic dietary risk assessment.

The NOEL for developmental neurotoxicity is lower than that of the fetal NOEL from the prenatal developmental toxicity study for eight out of nine pesticides tested, and demonstrates an equivalent NOEL for one (CHEMICAL X). Additionally, for the solvent TGME, the offspring NOEL from the gavage-dosed developmental neurotoxicity study is less than the fetal NOEL from the gavage prenatal developmental toxicity study. Making the same comparison between the DNT study and the two-generation reproduction study, it is noted that the offspring NOEL for the developmental neurotoxicity study is lower than the offspring NOEL for the reproduction study for six of the nine pesticides (aldicarb, carbaryl, DEET, emamectin, fipronil, and CHEMICAL X) and equivalent for one (chlorpyrifos). (No reproduction study was performed for TGME.) In light of the fact that the developmental neurotoxicity study measures neurobehavioral and histopathological endpoints that are not examined in the either the prenatal developmental or reproductive toxicity studies, this tendency is not unexpected, even though the animals in the reproduction study are treated over a longer period of time than those on the developmental neurotoxicity study. (Since the two remaining solvent chemicals, 1,1,1-TCE and isopropanol, did not demonstrate effects on the offspring that could be interpreted as developmental neurotoxicity, they were not compared in a similar manner.)

The developmental neurotoxicity NOEL for the offspring was less than or approximately equal to the acute and/or subchronic neurotoxicity NOELs in adult animals for six of the nine pesticides (carbaryl, carbofuran, chlorpyrifos, molinate, DEET, and emamectin). Overall, in two of nine cases (carbaryl and emamectin), the NOEL for developmental neurotoxicity was lower than or equal to that for any adult or offspring endpoint from the prenatal developmental, reproduction, or neurotoxicity studies.

While such limited quantitative comparisons (of NOELs) are useful, qualitative aspects of the study results can also provide a valuable basis of comparison. Even with similar NOELs for maternal and offspring toxicity in the developmental neurotoxicity study, evaluation of the findings in the context of the entire toxicological database may elicit additional concern regarding effects observed in the offspring. For example, in the situation where the NOELs for maternal versus offspring outcomes are equivalent in the developmental neurotoxicity study, but the alterations in the adults are characterized as transient while effects observed in the offspring are indicative of alterations in developmental processes which may have long-term consequences, there may be additional concern for risk to infants and children. An in-depth comparison of this nature would need to include consideration of the entire database for any chemical and could not be adequately undertaken for the chemicals evaluated in this paper, due to constraints of time and resources.

It is recognized that the conclusions drawn from this initial retrospective survey of developmental neurotoxicity data must be examined in light of the many confounding factors that may have contributed to the study results and conclusions. Some of these factors are common to many or all of the studies, such as the influence of dose selection on determination of the NOEL, inaccuracies or inconsistencies in the conversion of dietary or inhalation dose levels to mg/kg/day values, a lack of knowledge regarding actual exposure of the chemical to the offspring *in utero* or via the milk (pharmacokinetic data), or differences in the endpoints examined for the various protocols (for example, the timing of measurements, variations in laboratory procedures, missing or inadequate assessments of any particular endpoint). Some factors are specific to a chemical or a particular study protocol. These might include utilization of knowledge on the chemical to aid in the selection of tests to assess learning and memory or of the most appropriate species for testing. It is also acknowledged that the conclusions of the studies, as well as the endpoints selected for risk assessment, are often issues of contention between the Agency and the regulated community. There are on-going, unresolved controversies regarding some of the studies presented in this paper as well as some of the Agency decisions cited in this analysis. It is not intended that this document provide a detailed description of all such issues, nor that it provide a forum to resolve them.

General issues raised by the analysis of the DNT studies

A number of topics and issues germaine to the conduct of developmental neurotoxicity testing and data analysis have arisen in the review of the twelve studies.

• Route of administration

As for any toxicology study, the most appropriate route of administration for the evaluation of developmental neurotoxicity is that which is most similar to anticipated human exposure. For most pesticides, most particularly food-use pesticides, oral administration (dietary, drinking water, or gavage) is generally considered the most appropriate route, although in some cases dermal or inhalation studies may be more appropriate. There are, of course, advantages and disadvantages for every route of administration, and the effect of route on study design, conduct, and interpretation must be considered (Francis, 1994). For example, an advantage of gavage administration is that the exact measurement of the administered chemical is known and can be adjusted to body weight throughout the study. However, gavage dosing may be more irritating to the stomach. Additionally, gavage administration provides a discrete bolus daily dose of test substance while rats on a dietary study eat throughout each night and receive lower doses over multiple hours; it can be argued that neither of these scenarios is similar to human exposure to a test substance. Likewise, protocols that utilize dermal or inhalation administration (which generally expose the animals to discrete 4- to 6-hour daily exposure periods that are designed to mimic worker exposure scenarios) may not have a direct corollary to human exposure in a residential setting either.

A separate but important consideration is that in this study protocol, maternal animals are exposed to the test substance and the route of exposure is described in terms of these adult females; however, the offspring are the primary target of concern. The exposure to the offspring should be a more important consideration in the planning and implementation of these studies. This topic is clearly related to the issues of direct postnatal dosing of the pups (including the interpretation of data from dietary studies where pups begin to consume test substance in the feed prior to weaning and in that way are receiving a comparably large dose of test substance), and the need for pharmacokinetic data that allow some estimate of exposures *in utero* and/or via the milk or feed.

For one of the pesticides (DEET), the most significant anticipated human exposure is by dermal route, although oral (dietary) administration was used in the developmental neurotoxicity study. Similarly, for the toxic substances, gavage administration of the test substance was used, although the anticipated route of human exposure is expected to be via inhalation. For DEET, an adequate data base of oral toxicology studies have been generated, facilitating interpretation of the study results in context of the entire toxicology database. However, the developmental, reproductive, and adult neurotoxicology data examined for the three OPPT solvent chemicals included in this survey were primarily generated via inhalation dosing protocols, thereby limiting the ability to place the results of the solvent developmental neurotoxicity studies into a meaningful toxicological context.

• Duration of treatment

The OPPTS 870.6300 developmental neurotoxicity guideline specifies that the dams be treated from gestation day 6 through lactation day 10. However, nervous system development in the rat continues postnatally past that point. It has been suggested that in order to more fully assess the nervous system and functional development in the rat, and to provide a better model for insults to human nervous system development which also continues postnatally, treatment should be extended, at least to weaning (PND 21). To date, few studies have been conducted in this manner, and there are no definite conclusions that can be derived from the available data. In the analysis of those studies that were conducted with dosing extended through to the weaning of the pups (DEET, emamectin, TGME, and isopropanol), there were no obvious significant alterations in response that could be attributed to late lactation dosing of the offspring via the diet and/or maternal milk. However, this observation is not conclusive. Of these four chemicals, only emamectin produced observations of developmental neurotoxicity, but there were no comparative data from a study that terminated emamectin treatment at postnatal 10.

• Combined protocols

As stated previously, for two of the pesticides evaluated, the developmental neurotoxicity studies were conducted as combined study protocols, either as a segment of a two-generation reproduction study (DEET) or in conjunction with a prenatal developmental toxicity study (CHEMICAL X). The use of combined studies to evaluate neurodevelopmental deficiencies is more complex to plan and perform, but may result in a more efficient use of study time and resources and is encouraged by the Agency, as stated in the guideline for reproduction and fertility effects [OPPTS 870.3800(b)]. A careful review of the DEET study design reveals that all evaluation of neurological toxicity was conducted on animals which were adults (at least 40 weeks of age) at the time of testing, even though they had been exposed to the test substance from conception through termination. No testing was performed on the offspring prior to weaning nor in early maturation. Additionally, some parameters, such as the age of sexual maturation, were not evaluated. For these reasons, it is difficult to compare the findings from the DEET study with others conducted according to the standard developmental neurotoxicity testing guideline. Nevertheless, it is recognized that it would have been possible to perform all standard testing recommended in the DNT protocol on the 2-generation reproduction study protocol designed for DEET, and that an expanded study design of this sort could be useful.

• Cholinesterase inhibition

The generic DNT guideline does not specify that cholinesterase activity be evaluated for chemicals known to produce effects on these enzymes. Nevertheless, observations on cholinesterase inhibition can be used to determine the adequacy of the dose levels selected and to assist in the interpretation and evaluation of effects noted in the offspring.

In the aldicarb study, blood and brain cholinesterase activity data were measured in pups at postnatal days 4, 10, and 11. Neither fetal nor postnatal cholinesterase measurements were collected in any other study examined in this retrospective analysis; however, these data were examined in a companion study for chlorpyrifos. In that study, maternal rats were administered the test chemical using the same treatment regime as in the developmental neurotoxicity study (Mattsson et al., 1998). Briefly, blood and milk samples were collected from dams and blood samples were collected from offspring, for the determination of levels of chlorpyrifos and two metabolites (chlorpyrifos-oxon and 3,5,6-trichloro-2-pyridinol [TCP]), on gestation day 20 and lactation days 1, 5, and 11. Cholinesterase activity was measured in blood (plasma and RBC) and tissue (brain and heart) samples taken from dams and offspring on gestation day 20 and lactation days 1, 5, 11, 22, and 65 (pups only). Measurable levels of chlorpyrifos and/or its metabolites were demonstrated in dam and offspring blood during gestation and/or lactation for all treated groups in a dose-related pattern. Notably, milk concentrations of chlorpyrifos on lactation day 1 and 5 were at least 10-fold greater than blood concentrations in all dose groups. Cholinesterase was inhibited in dams of all treated groups with approximately the same profile as in the developmental neurotoxicity study (plasma ChEI at the low-dose, RBC and brain ChEI at the mid- and high-doses); however, inhibition of cholinesterase activity in fetuses and pups was only observed at the high-dose. These data provide valuable information that support and more clearly define the adequacy of dosing and the resultant findings of the developmental neurotoxicity study with chlorpyrifos.

• Pharmacokinetic data

For developmental neurotoxicity studies reviewed by the Agency, there is generally a lack of knowledge regarding actual exposure of the chemical to the offspring *in utero* or via the milk. Pharmacokinetic data, which might assist in this determination, are not addressed in the standard developmental neurotoxicity study guideline and were only available (in supplementary studies) for two of the chemicals reviewed (aldicarb and chlorpyrifos). These data (not presented in detail in this paper) were valuable in establishing exposure of the pups to the test substances, and interpreting the study results. For chlorpyrifos, there was bioconcentration in maternal milk, and presence of the chemical and/or its metabolites was demonstrated in both fetuses and pups. However, for aldicarb, low demonstrable levels of the chemical in maternal milk, along with the lack of cholinesterase inhibition in pups, indicated that the pups were receiving very little test substance during the postnatal period. This suggests that a developmental neurotoxicity protocol which includes direct postnatal exposure to the offspring may be necessary to adequately evaluate the developmental neurotoxic potential of some chemicals. Although no standardized testing guideline for postnatal dosing has been developed by the Agency, a study with chlorpyrifos by Moser and Padilla (1998) is an example of a very useful protocol that includes direct dosing to offspring. An additional consideration that speaks to the need for pharmacokinetic data is that they could be useful in extrapolating from rat toxicity studies to effects in humans, where more of neural development is taking place during gestation.

• Simple morphometric analysis

Since developmental insult may alter the growth of specific regions of the brain in the absence of overt lesions such as those characteristic of adult neuropathology, the developmental neurotoxicity guidelines require that measurements be made of several brain regions. Simple measurements at homologous locations within the developing brain can detect alterations in the range of 10-20% (Rodier and Gramann, 1979). Comprehensive measurements of all brain regions is time- and cost-prohibitive and the number of brain regions comprising an adequate assessment remains controversial (Jensen, 1995). Consequently, the guidelines specify that a morphometric approach be used, providing the flexibility to make appropriate use of the professional judgement, subjective histological assessment, and established protocols of the neuropathologist conducting the study for the selection of the most appropriate approach to measurement and selection of regions to measure. The detection of morphometric alterations in half the cases in which this approach was used argues for its value in detecting developmental insult to diverse regions resulting from developmental exposure to chemical insult.

• Age-related susceptibility

When developmental neurotoxicity is seen at dose levels which are not toxic to the maternal animal in the developmental neurotoxicity study, this is not necessarily evidence of greater susceptibility of the perinatal animal as compared to the adult. In this study, the focus is on the evaluation of the offspring, and evaluation of the dams is not as detailed or extensive. Careful consideration of the database in its entirety may provide additional information that could place the neurodevelopmental findings in perspective, or due to variations in dosages and/or endpoints examined, such data may be of only limited assistance. Nevertheless, qualitative comparisons of adult and offspring response to treatment also need to be carefully evaluated in the context of the entire toxicological database in order to provide an adequate and accurate interpretation of the study findings and to identify areas of concern regarding the comparative neurotoxicological response of adults and offspring to chemical insult.

<u>The use of the developmental neurotoxicity study to select endpoints for risk assessment</u>

In September, 1996, a Health Effects Division guidance document entitled *Hazard Identification - Toxicology Endpoint Selection Process* was presented to the Scientific Advisory Panel for consideration. This document has since been finalized (August 11, 1998) and describes the current process by which endpoints are selected for long-term (chronic) and less-than-lifetime risk assessment in the Hazard Identification Assessment Review Committee (HIARC). This includes endpoints for acute and chronic dietary risk assessment, and for short-, intermediate-, and long-term occupational or residential risk assessment for dermal and inhalation exposures. For each exposure scenario, guidance is provided for the evaluation of toxicity studies that are relevant for use (due to similar route and duration of the study to the exposure of interest) and selection of appropriate endpoints for hazard identification (doses and endpoints that best define the potential hazard in association with the exposure scenario).

At the time that this guidance document was drafted, there were few developmental neurotoxicity studies available for consideration; therefore, the document does not specifically address the use of this study for the selection of endpoints for risk assessment. It is assumed that developmental neurotoxicity effects in animal studies indicate the potential for altered neurobehavioral development in humans, although the specific effects may not be the same. Therefore, as stated in the Guideline for Neurotoxicity Risk Assessment (1998), "*when data suggesting adverse effects in developmental neurotoxicity studies are encountered for particular agents, they should be considered in the risk assessment process.*" The following guidance is proposed for addition to the *Toxicology Endpoint Selection Process* document.

Developmental neurotoxicity endpoints (effects on offspring) can be used in acute dietary risk assessment, in spite of the apparent potential for multiple *in utero* and/or postnatal exposures to offspring during the maternal dosing period (GD6-PND10 = 25 days), since it is assumed that these findings could result from a single exposure to the chemical. This assumption is supported by numerous reports (of developmental neurotoxicity elicited following a single maternal or perinatal exposure) in the peer-reviewed literature. These included neurobehavioral and/or neuropathological alterations of the offspring in studies with halothane or nitrous oxide (Rodier and Koeter, 1986), methyl mercury (Sager et al., 1984), 5-azacytidine (Rodier et al., 1979), valproic acid (Rodier, 1996), ethanol (Goodlett et al., 1989; Gavin et al., 1994), methylazoxymethanol (Rodier et al., 1991; Gavin et al., 1994), acetylsalicylic acid (Vorhees et al., 1982), and alkyltins (Reiter et al, 1981; Cook et al, 1984; Stine et al., 1988). Any treatment-related neurobehavioral or neuropathological evidence of alteration to offspring development is considered appropriate to use in acute dietary risk assessment. Should such an endpoint be selected, it is considered pertinent for all population subgroups that contain infants and children, not only for Females 13+, since it is not possible to differentiate whether the neurodevelopmental effect was the result of a single pre- or postnatal exposure. This differs from the situation in which a developmental endpoint is selected from a prenatal developmental toxicity study. In that case, the endpoint from the prenatal developmental toxicity study is used in the risk assessment for Females 13+, and another endpoint is selected if available, for the risk assessment for other populations for which gestational exposure will not be applicable (e.g., adult males). The use of the developmental neurotoxicity endpoint, assuming that it represents the lowest NOAEL in the database, is generally judged to be protective for all populations. The General Population dietary risk assessment subgroup, which includes infants and children of various age categories, also includes adult males; however, it is generally not considered necessary to select a different and specific endpoint for a risk assessment for the subpopulation of adult males.

Due to the detailed observations on maternal animals, the developmental neurotoxicity study might also be a good source for an acute dietary endpoint which is based upon clinical observations or other toxicity in the dams which are observed after a single dose of the test substance.

The maternal dosing duration on the developmental neurotoxicity study is approximately 25 days, which makes it a candidate, along with numerous other studies of short duration (e.g., 21-day dermal, subchronic, or prenatal developmental studies), for use in short- and/or intermediate-term risk assessment. The duration of exposure for each of these categories is *1-to-7-days* and *1-week-to-several-months*, respectively. Should the chemical evaluated in the developmental neurotoxicity study be dosed via the dermal (or inhalation) route, or should no other dermal (or inhalation) studies be available, the developmental neurotoxicity study may be appropriate as a source of endpoints (offspring or maternal) for short- or intermediate-term risk assessment. In this case, the assumption is made that the finding may have been the result of multiple doses, as could occur, for example, when a bioaccumulation of the test chemical in maternal tissues is necessary to reach a developmentally toxic concentration. Route to route extrapolation of dose levels may be necessary.

Although no calculations of short- or intermediate-term dietary risk assessment for infants and children are currently conducted, the developmental neurotoxicity study should be considered an appropriate study for use in endpoint selection, if in the future such a risk assessment were to be conducted by OPP.

Although the dosing duration on a developmental neurotoxicity study does not approximate a lifetime (chronic) exposure to a chemical, it may still be appropriate in some instances to select maternal or offspring endpoints from this study for long-term dietary (RfD) calculations and/or for dermal and/or inhalation occupational and residential risk assessments. This might occur, for instance, if no other appropriate endpoint can be identified in long-term studies, or if the developmental neurotoxicity NOEAL is less than the NOAELs derived from the long-term studies.

The selection of any endpoint for risk assessment for each chemical, whether it be from the developmental neurotoxicity study or any other study in a chemical database, requires sound scientific judgement, and all information and rationale that contribute to the final outcome should be thoroughly documented by HED.

DISCUSSION/CONCLUSIONS

Developmental exposure to agents can produce neurotoxic effects that differ qualitatively and quantitatively from those produced by adult exposure (Riley & Vorhees, 1986; Kimmel et al., 1990). Several well-documented examples of chemical substances that produce developmental neurobehavioral effects in both animals and humans and occur at levels that do not cause toxicity in adults include environmental lead, methylmercury, PCBs, ethanol (fetal alcohol syndrome), and certain antiepileptic agents (reviewed in Kimmel et al., 1990). There is a need for both adult and developmental neurotoxicity evaluations in EPA's toxicity testing strategy to characterize the hazards and the dose-response relationships for risk assessment.

The developmental neurotoxicity study protocol (OPPTS 870.6300) includes unique endpoints which are not examined in any other standard toxicity testing protocol, enabling the detection of effects on nervous system development of the offspring following pre- and/or postnatal exposure.

In this initial retrospective analysis, nine pesticides and three solvents were examined. The methods and results of the developmental neurotoxicity studies for these chemicals were evaluated, and the findings were placed into the context of the data from guideline prenatal developmental, reproductive, and neurotoxicity studies. NOELs from these studies, as well as the NOELs from studies used to provide endpoints for acute and chronic dietary risk assessment were compared.

Due to the limited number of studies examined in each of several chemical classes, no conclusions could be drawn regarding the correlation of study findings to chemical class. The class with the highest representation was the carbamates, with four chemicals studied (aldicarb, carbaryl, carbofuran, and molinate).

For the group of chemicals being reviewed, positive findings in the offspring were noted across studies for all types of observations recorded: developmental landmarks, behavioral/functional observations, sensory function, motor activity, learning and memory, brain weight, and/or neuropathology. In addition, a striking feature of the results of these developmental neurotoxicity studies was the high degree of concordance between functional and structural assessments. For five of the six pesticides for which morphometric analysis was conducted, alterations were identified in both behavior and brain morphology. This high frequency of concordance is critical to ascertaining the biological plausibility of the potential action of these pesticides on developmental events critical to behavioral maturation. The detection of concordance in the developmental neurotoxicity studies is likely due to a variety of biological and methodological factors, including the focus of these studies on developmental profiles derived from assessments at multiple time points. This supports the need for assessing a variety of functional and developmental neurobehavioral and neuropathological endpoints to screen for effects on nervous system development.

The evaluation of effects on neurological development, as currently embodied in the developmental neurotoxicity study, is a sensitive indicator of toxicity to offspring. Even for the small number of chemicals examined, the developmental neurotoxicity study often identified a lower NOEL than other standard guideline animal studies that address other types of effects on perinatal organisms or on adults. This study appears to be a valuable tool in the characterization of hazard to infants and children.

It is recommended that data from developmental neurotoxicity studies that are received and reviewed by the OPPTS in the future should be evaluated and utilized in an on-going effort to expand the analysis presented in this paper. More focused and detailed analyses of many elements in these studies could be useful, although they are resource-intensive and difficult to achieve in a busy regulatory environment. Developmental neurotoxicity study data that are received by other Agency offices should also be considered for inclusion, as appropriate. (For example, a perchlorate developmental neurotoxicity study is currently being evaluated by the EPA Office of Research and Development.)

Agency efforts have been initiated to reconsider the standard protocol currently used for developmental neurotoxicity testing. These efforts should continue, culminating in a proposal of revisions to the guideline currently in use. The Agency should, as part of this process, continue to participate in further evaluation and standardization of the endpoints examined in a developmental neurotoxicity study and participate fully in international discussions regarding the developmental neurotoxicity protocol (including the OECD developmental neurotoxicity guideline development and the recommendations of the Endocrine Disruptor Screening and Testing Advisory Committee).

It has been proposed in this paper that the HED document *Hazard Identification: Toxicology Endpoint Selection Process* (1998) should be revised to include consideration of developmental neurotoxicity endpoints for risk assessment. The use of such endpoints, when appropriate, should be implemented immediately by the Hazard Identification Assessment Review Committee.

Table 4A. Results of developmental neurotoxicity studies received/reviewed by OPPTS: maternal toxicity

Chemical	Doses Tested (mg/kg/day)	Effect Levels	Gestation BW	Gestation FC	Lactation BW	Clinical observations/FOB	Gross pathology
Aldicarb	0.05, 0.10, 0.30	0.10				Plasma ChEI on GD7	
		0.30	↓ BW/BWG		↓ BW PND4	Tremors, salivation, lacrimation, stained fur, hunched posture, gait abnormalities, ear flicking, lip smacking, fewer rears, miosis, and/or mild ataxia; blood ChEI	
Carbaryl	0.1, 1.0, 10	10	↓ BWG			Tremors; ataxic gait; pinpoint pupils; blood, RBC, and brain ChEI	
Carbofuran	1.7, 6.9, 31	6.9	↓ BWG	↓ FC			
		31	↓ BW/BWG	↓ FC			
Molinate	1.8, 6.9, 26.1	26.1	↓ BW/BWG	↓ FC	↓ BW/BWG		
DEET	22.5, 90, 225	225	↓ BW/BWG				
Emamectin	0.1, 0.6, 3.6/2.5	>3.6/2.5 a					
Fipronil	0.05, 0.9, 18.5	18.5	↓ BW/BWG	↓ FC		Alopecia	
Chlorpyrifos	0.3, 1, 5	0.3				Plasma and RBC ChEI on GD20	
		1				Plasma, RBC, and brain ChEI on GD20	
		5				Fasciculations, hyperpnea, hyperreactivity; plasma, RBC, and brain ChEI on GD20	
CHEMICAL X	40, 125, 400	125		↓ FC		Salivation, rales	
		400	↓ BW/BWG	↓ FC	↑ BWG	Salivation, rales, ataxia, urine-stained fur, ↓ motor activity	
1,1,1-TCE	75, 250, 750	>750 a					
TGME	300, 1650, 3000	3000					↓ kidney weight
Isopropanol	200, 700, 1200	1200				mortality (1/35)	

a = No maternal toxicity observed.

Table 4B. Results of developmental neurotoxicity studies received/reviewed by OPPTS: offspring toxicity

Chemical	Doses Tested (mg/kg/day)	Effect Levels	Physical development and behavioral/functional observations	Motor activity	Sensory function and auditory startle habituation	Learning and memory	Brain weight and Neuropathology
Aldicarb	0.05, 0.10, 0.30	0.10	↓ pup wt; ↓ hindlimb grip strength and splay in ♀s PND 35	↓ MA in ♂s PND 17;↑ MA in ♂s PND 60			e
		0.30	↓ pup wt; ↓ rears in ♂s PND 35 and 63, ♀s PND 35; ↓ hindlimb grip strength and splay in ♀s PND 35 and ♂s PND 63; ↓ forelimb grip strength in ♂s PND 63; ↓ latency to 1st step in ♂s PND 63	↓ MA in ♂s PND 17;↑ MA in ♂s PND 60	↑ latency to heat stimulus on first trial for ♂s on PND 63		
Carbaryl	0.1, 1.0, 10	10					altered morphometric measurements (forebrain and/or cerebellum) in ♂s and ♀s on PND 11 and 60 d
Carbofuran	1.7, 6.9, 31	6.9 31 a	↑ mortality PND 0-4; ↓ pup wt; delayed pinna unfolding, incisor eruption, eye opening, and sexual maturation; delayed swimming angle development			↑ in time trials on PND 25 (♂s and ♀s) and 30 (♂s only) in water Y maze	↓ absolute brain weight on PND 11(with severe↓ pup wt) e
Molinate	1.8, 6.9, 26.1	1.8			↓ startle amplitude in ♀s on PND 23		
		6.9			↓ startle amplitude and latency in ♀s on PND 23		↓ morphometric measurements (cerebellum) in ♂s and ♀s on PND 12

Table 4B. Results of developmental neurotoxicity studies received/reviewed by OPPTS: offspring toxicity

Chemical	Doses Tested (mg/kg/day)	Effect Levels	Physical development and behavioral/functional observations	Motor activity	Sensory function and auditory startle habituation	Learning and memory	Brain weight and Neuropathology
		26.1	↑ mortality; ↓ pup wt; delayed sexual maturation; ↓ swimming ability in straight channel in ♂s and♀s on PND 21	↓ mean MA in ♂s on PND 14;↑ mean MA in ♂s on PND 22 and 60	↓ startle amplitude in ♂s PND 23; ↓ startle amplitude in ♀s PND 23 & 61	↓ % successful trials during learning and memory phases at PND 21-24 in water Y maze	↓ brain weight on PND 12 and 63 in ♂s and ♀s, ↓ brain length on PND 12 ♂s and ♀s, and ↓ brain width on PND 12 in ♀s; ↓ morphometric measurements (cortex, hippocampus, cerebellum) in ♂s and ♀s on PND 12 and 63
DEET	22.5, 90, 225	225		↑ MA at beginning of testing session			e
Emamectin	0.1, 0.6, 3.6/2.5	0.6		↓ open field MA in ♀s on PND 17			
		3.6/2.5	↓ pup wt; head/body tremors, hindlimb extension/splay; delayed developmental landmarks	↑ MA at PND 13; ↓ MA on PND 17; ↓ MA in ♀s on PND 59	↓ auditory startle reflex at PND 22 & 59		↓ absolute brain weight (with ↑ relative brain weight) at PND 60 in ♂s and ♀s
Fipronil	0.05, 0.9, 18.5	0.9 b	↓ pup wt				
		18.5 c	↓ pup wt; ↓ survival; delayed incisor eruption and sexual maturation; delayed swimming angle development	↑ MA in ♀s on PND 17	↓ auditory startle response at PND 22	↑ time in water maze in ♀s on PND 24	↓ absolute brain weight at PND 11 and 60 in ♂s and ♀s (↑ relative brain weight at PND 11 in ♂s and ♀s)
Chlorpyrifos	0.3, 1, 5	5	↓ pup and F1 adult BW; ↓ postweaning FC; ↓ survival; delayed pinna unfolding and sexual maturation		↓ auditory startle peak response at PND 23 and 62; ↑ latency to peak response at PND 62		↓ absolute brain wt (and↑ relative brain wt) in ♂s and♀s on PND 12; ↓ morphometric measurements (cerebellum, cortex, caudal-putamen, hippocampus, and/or parietal) at PND 12 and 62
CHEMICAL X	40, 125, 400	400	↓ pup wt	↓ MA in ♂s on PND 18			
1,1,1-TCE	75, 250, 750	>750 f					

Table 4B. Results of developmental neurotoxicity studies received/reviewed by OPPTS: offspring toxicity

Chemical	Doses Tested (mg/kg/day)	Effect Levels	Physical development and behavioral/functional observations	Motor activity	Sensory function and auditory startle habituation	Learning and memory	Brain weight and Neuropathology
TGME	300, 1650, 3000	1650	↓ pup BWG				
		3000	↓ pup BWG		↑ maximum startle amplitude in ♂s on PND 22 and 60; ↓ response time in ♂s on PND 60		
Isopropanol	200, 700, 1200	>1200 f					

a = The same findings were observed in offspring at both the mid- and high-dose levels.
b = Developmental toxicity LOEL.
c = Developmental neurotoxicity LOEL.
d = Morphometric analysis only performed on control and high-dose groups; low- and mid-dose assessments have been requested by the Agency.
e = Morphometric analysis not conducted.
f = No offspring toxicity noted.

Table 5. Prenatal developmental toxicity in rats

Chemical	Doses Tested (mg/kg/day) a	Maternal		Fetal	
		NOEL LOEL	Endpoint	NOEL LOEL	Endpoint
Aldicarb	0.125, 0.25., 0.5	0.125 0.25	↓ BWG/FC	0.125 0.25	↑ incidence of ecchymosis of the trunk; at 0.5 mg/kg:↓ fetal weight, ↑ dilation of lateral ventricles of the brain (with tissue depression)
Carbaryl	b	10 100	↓ BWG	10 100	↓ implantations, ↓ live fetuses
Carbofuran	1.0, 3,0, 8.0	1.0 3.0	↓ BWG	3.0 8.0	↓ fetal weight
Molinate	2.2, 35, 140	35 140	↓ BWG/FC; ↑ salivation/ dehydration; RBC ChEI	2.2 35	↑ runting
DEET	125, 250, 750	250 750	↓ BWG/FC mortality; ↓ BWG/FC; ↑ liver weight	250 750	↓ fetal weight
Emamectin	2, 4, 8	2 4	↓ BWG	4 8	↓ fetal weight; ↑ supernumerary ribs
Fipronil	1, 4, 20	4 20	↓ BWG/FC	$\geq$20 --	No developmental toxicity observed
Chlorpyrifos	0.1, 3.0, 15.0 c	0.1 3	plasma and RBC ChEI	$\geq$15 --	No developmental toxicity observed (fetal ChEI not assessed)
	0.5, 2.5, 15.0 d	<0.5 0.5	plasma ChEI; ↓ BW/FC at 2.5 mg/kg/day	2.5 15	↑ postimplantation loss (fetal ChEI not assessed)
CHEMICAL X	40, 125, 400	40 125	clinical signs: salivation, rales	125 400	↓ fetal weight
1,1,1-TCE	1000, 3000, 6000 ppm	1000 3000	↓ BWG/FC; hypoactivity, perioral wetness	3000 6000	↑ resorptions

Table 5. Prenatal developmental toxicity in rats

Chemical	Doses Tested (mg/kg/day) a	Maternal		Fetal	
		NOEL LOEL	Endpoint	NOEL LOEL	Endpoint
TGME	652, 1250, 2500, 5000	1250 2500	at 2500 mg/kg/day: ↓ BWG/FC; at 5000 mg/kg/day: mortality (1/25); ↓ BWG/FC; salivation, ataxia; ↓ motor activity; impaired righting reflex	625 1250	at 1250 mg/kg/day: delayed ossification; at 2500 mg/kg/day: ↓ fetal weight, delayed ossification; at 5000 mg/kg/day: ↑ resorptions, ↓ fetal weight, delayed ossification
Isopropanol	400, 800, 1200	400 800	mortality	400 800	↓ fetal weight

a = Studies conducted by gavage with the exception of 1,1,1-TCE, which was administered by inhalation.
b = Carbaryl developmental and reproductive toxicity assessment from JMPR manuscript (1996).
c = Fischer 344 rats (Oullette, 1983).
d = Sprague-Dawley rats (Rubin, 1987).

Table 6. Multi-generation reproduction study in rats

Chemical	Doses Tested (mg/kg/day) a	Parental		Offspring	
		NOEL LOEL	Endpoint	NOEL LOEL	Endpoint
Aldicarb	0.1, 0.4, 0.7, 1.4 ♂ 0.2, 0.4, 0.9, 1.7 ♀ (2, 5, 10, 20 ppm)	0.4♂ 0.7♂ 0.4♀ 0.9♀	↓ BW; plasma and RBC ChEI	0.7♂ 1.4♂ 0.9♀ 1.7♀	↓ pup weight; ↓ survival
Carbaryl	b	100 200	↓ maternal BWG	≥200 >200	no offspring toxicity
Carbofuran	1, 10 (20, 100 ppm)	1 10	↓ premating BW/FC; ↓ gest. BWG	1 10	↓ pup survival PND 0-4; ↓ PND 21 pup weight
Molinate c	0.4, 0.8, 1.3 ♂ 1.9, 4.7, 28.8 ♀ (5, 10, 15 ppm ♂ 20, 50, 300 ppm ♀)	<0.4 ♂ 0.4 ♂ <1.9 ♀ 1.9 ♀	↓ brain weight; at 0.8/4.7 (♂/♀) mg/kg/day:↑ abnormal sperm and ↓ cauda weight; histopathological lesions in adrenal and ovary; at 1.3/28.8 (♂/♀) mg/kg/day:↓ BWG/FC; ↓ mating; ↓ uterus and epididymis weight	0.4 ♂ 0.8 ♂ 1.9 ♀ 4.7 ♀	↓ F2b ♀ brain wt.;↓ testes and spleen weight; delayed vaginal opening; at 1.3/28.8 (♂/♀) mg/kg/day:↓ pup weight and survival; ↓ spleen, ovary, and/or thymus weight
DEET	25, 100, 250 (500, 2000, 5000 ppm)	<25 25	gross and histopathological kidney lesions	≥250 >250	no offspring toxicity
Emamectin	0.1. 0.6, 3.6/1.8 d	0.6 1.8	↓ BWG; neuronal degeneration in P and F1♂s and♀s (brain and spinal cord)	0.6 1.8	↓ fertility/fecundity indices; tremors and hind limb extension in F1/F2 pups
Fipronil	0.25, 2.54, 26.0 (3, 30, 300 ppm)	0.25 2.5	↑ thyroid and liver weight; ↓ pituitary weight; thyroid histopathology	0.25 2.5	↑ clinical signs; ↓ litter size; ↓ BW; ↓ pre- and postnatal survival; delays in physical development
Chlorpyrifos	0.1, 1.0, 5.0	0.1 1.0	plasma and RBC ChEI; histopathology of adrenal in♀s; at 5.0 mg/kg/day: brain ChEI	1.0 5.0	↓ pup weight; ↓ survival (ChEI not assessed in pups)

Table 6. Multi-generation reproduction study in rats

Chemical	Doses Tested (mg/kg/day) [a]	Parental		Offspring	
		NOEL LOEL	Endpoint	NOEL LOEL	Endpoint
CHEMICAL X	5.7, 25.1, 152.4 ♂ 6.8, 29.4, 172.4 ♀ (94, 410, 2500 ppm)	5.7 ♂ 25.1 ♂ 6.8 ♀ 29.4 ♀	↑ liver weight; hepatocellular hypertrophy	≥197.9 ♂ >197.9 ♂ ≥325.1♀ >325.1♀ [e]	no offspring toxicity
1,1,1-TCE	NR	NR			
TGME	NR	NR			
Isopropanol	100, 500, 1000	100 500	↑ liver weight and histopathology in ♂s and♀s; at 1000 mg/kg/day: ↑ ♂ mating index	100 500	↑ F1 postnatal survival

a = Route of administration was dietary for all studies except isopropanol, which was administered by gavage; dietary dose levels expressed as ppm were converted to mg/kg/day.
b = Developmental and reproductive toxicity assessment from JMP manuscript (1996).
c = An additional female-only reproduction study identified a NOEL/LOEL of 0.34/2.9 mg/kg/day, based on ↓ brain weight, ↓ fecundity, ↑ vacuolation/hypertrophy of ovary.
d = Dietary level at the high dose was reduced on GD 0 of the second P mating, due to observations of tremors in F1 pups.
e = Maximum dose values determined at any time point during the study.
NR = Not required (no reproduction study was conducted).

Table 7. Neurotoxicity profile: Acute neurotoxicity studies in rats

Chemical	Doses (mg/kg) a	NOEL LOEL	Endpoints at LOEL
Aldicarb	0.05, 0.1, 0.5	<0.05 0.05	plasma ChEI; also at 0.1 mg/kg: blood ChEI; also at 0.5 mg/kg: blood and brain ChEI tremors, lacrimation, salivation, ↓ temperature, ↑ respiration, ↓arousal, activity and reactivity, ↓ fore- & hindlimb grip strength; ↓ motor activity
Carbaryl	10, 50, 125	<10 10	plasma, RBC, whole blood, & brain ChEI; ↓ motor activity; also at 50 &/or 125 mg/kg: ↓ BWG/FC, tremors, salivation, ataxic gait, ↓ body temperature, ↓ arousal, ↓ fore- & hindlimb grip strength,↓ motor activity
Carbofuran	NS	NS	NS
Molinate	25, 100, 350	< 25 25	↓ BWG; ↓ FC; ↓ activity, RBC & brain ChEI; ↑ landing foot splay; ↑ time to tail flick
DEET	50, 200, 500	50 200	↓ vertical motor activity; at 500 mg/kg (HDT): ↓ vertical and horizontal motor activity, piloerection, vocalization, ↑ response time to heat stimulus
Emamectin	27.4, 54.8, 82.2	<27.4 27.4	salivation, tremors, ataxia, bradypnea, loss of righting reflex, ↓ activity; histopathological lesions in brain, spinal cord, sciatic nerve
	0.5, 2.5, 5, 10, 25	5.0 10.0	tremors and irritation; at 25 mg/kg (HDT): clinical signs, neuronal lesions (FOB and motor activity testing were not conducted)
Fipronil	0.5, 5, 50	0.5 5	at 7 hours postdose:↓ hindlimb splay in both sexes; ↓ body temperature in ♂s (1993)
	2.5, 7.5, 25	2.5 7.5	↓ BWG; ↓ FC/FE; ↓ hindlimb splay in ♂s at 7 hrs postdose;↓ grooming in ♀s at 14 days postdose; at 25 mg/kg:↑ unusual behavior,↓ hindlimb splay,↑ grip strength,↓ body temperature, ↓ activity in ♀s (1997)
Chlorpyrifos	10, 50, 100	10 50	↓ BW, ↓ motor activity, cholinergic clinical signs; at 100 mg/kg (HDT) in ♀s: inability to perform the landing hind leg splay, ↓ grip strength
CHEMICAL X	NS	NS	NS
1,1,1-TCE	4000 ppm b	<4000 4000	↓motor activity in♂s (slight) and♀s on day 4
	1000, 2000 ppm b	<1000 1000	altered evoked potential and EEG component of somatosensory evoked potential; at 2000 ppm: greater severity
TGME	NS	NS	NS

Table 7. Neurotoxicity profile: Acute neurotoxicity studies in rats			
Chemical	Doses (mg/kg) a	NOEL LOEL	Endpoints at LOEL
Isopropanol	500, 1500, 5000, 10000 ppm c	1500 5000	transient narcosis (also at 10000 ppm)

NOEL and LOEL are expressed as mg/kg/day, except for inhalation studies
a = Studies administered by gavage unless otherwise specified.
b = Inhalation dosing: 6 hr/day for 4 days.
c = Inhalation dosing: 6 hours.
NS = Not submitted to the Agency.

Table 8. Neurotoxicity profile: subchronic neurotoxicity studies in rats

Chemical	Doses (mg/kg/day) a	NOEL LOEL	Endpoints at LOEL
Aldicarb	0.05, 0.2, 0.4 (gavage)	<0.05 0.05	Pinpoint pupils in ♂s; blood & brain ChEI; additionally, at 0.2 &/or 0.4 mg/kg: tremors, salivation, ↓ tail pinch response, ↓ fore- & hindlimb grip strength, ↓ body temperature, ↓ motor activity, ↑ rate of habituation of MA
Carbaryl	1, 10, 30 b	1.0 10.0	plasma, RBC, whole blood, & brain ChEI; tremors, gait alterations, pinpoint pupils, salivation, reduced extensor thrust, ↓ pinna reflex, ↓ rearings, ↓ vocalizations, ↓ body temperature, ↓ forelimb grip strength; also at 30 mg/kg: ↓ motor activity, hemorrhagic meninges
Carbofuran	2.4, 27.3, 55.3 ♂ 3.1, 35.3, 64.4 ♀ (50, 500, 1000 ppm)	<2.4 ♂ 2.4 ♂ <3.1♀ 3.1♀	↑ landing foot splay in ♀s; ↓ BWG; at 27.3/35.3 and 55.3/64.4 (M/F): exophthalmos, tremors, staggering gait, ataxia, splayed hindlimbs, loss of muscle control; ↓ BWG/FC; ↓ motor activity at HDT
Molinate	4.0, 11.7, 35.5 ♂ 4.5, 13.9, 41.0 ♀ (50, 150, 450 ppm)	<4.0♂ 4.0♂ <4.5♀ 4.5♀	brain ChEI; ↓ NTE activity (at MDT: RBC ChEI; at HDT: ↑ landing foot play, ↓ fore- and/or hindlimb grip strength, ↑ time to tail flick, ↑ motor activity in ♀s; ↓ brain weight; nerve fiber degeneration of sciatic and sural nerves in ♂s)
DEET c	22.5, 90, 225 (500, 2000, 5000 ppm)	90 225	↑ motor activity
Emamectin	0.25, 1, 5	1.0 5.0	↓ BWG;↓ FC; tremors, salivation, rough/soiled coats; effects on posture, rearing, gait, grip strength, mobility, righting reflex; neuropathology of the brain, spinal cord, and sciatic nerve; skeletal muscle atrophy in ♂s
Fipronil	0.03, 0.30, 8.89♂ 0.3, 0.35, 10.8♀ (0.5, 5, 150 ppm)	0.30♂ 8.89♂ 0.35♀ 10.8♀	FOB effects at wks 4, 9, & 13: in ♂s ↑ incidence of urination, exaggerated tail pinch response; in ♂s &♀s; ↑ incidence of exaggerated startle response; ↑ forelimb grip strength in ♀s (wk 13) (1993)
Chlorpyrifos	0.1, 1.0, 5.0, 15	$\geq$15 >15	no neurotoxicity noted (ChE activity was not measured)

Table 8. Neurotoxicity profile: subchronic neurotoxicity studies in rats

Chemical	Doses (mg/kg/day) a	NOEL LOEL	Endpoints at LOEL
CHEMICAL X	5.7, 25.1, 152.4♂ 6.7, 29.4, 172.4♀ (94, 410, 2500 ppm)	5.7♂ 25.1♂ 6.7♀ 29.4♀	↑ liver weight; hepatocellular hypertrophy and vacuolation; no neurotoxicity noted
1,1,1-TCE	200, 630, 2000 ppm d	630 2000	slightly ↓ forelimb grip strength
TGME	400, 1200, 4000 e	400 1200	↓ BW/FC
Isopropanol	100, 500, 1500, 5000 ppm d	1500 5000	↑ motor activity

NOEL and LOEL are expressed as mg/kg/day.
a = Dietary administration unless otherwise specified.
b = Administered by gavage.
c = Assessments conducted on F2 offspring of a two-generation reproduction/developmental neurotoxicity study.
d = Inhalation dosing: 6 hr/day for 13 weeks.
e = Administered in drinking water.
NS = Not submitted to the Agency.

Table 9. Risk assessment profile: studies and endpoints selected for risk assessment of pesticides										
Chemical	Acute					Chronic				
	Study	Endpoint	NOEL	UF	RfD	Study	Endpoint	NOEL	UF	RfD
Aldicarb	Human oral c	Sweating; plasma and RBC ChEI	0.01	10	0.001	Human oral c	Sweating; plasma and RBC ChEI	0.01	10	0.001
Carbaryl	Develop. neurotox.	Maternal FOB findings	1.0	100	0.01	Chronic & 5-wk dog	Plasma and brain ChEI	1.4	100	0.014
Carbofuran	Human oral c	RBC ChEI	0.5	30	0.016	Human oral c	RBC ChEI	0.05	100	0.0005
Molinate	Develop. neurotox.	↓ auditory startle in pups	<1.8	300	0.006	2 yr rat chronic	degeneration/demyelination of sciatic nerve	0.3	300	0.001
DEET	NR	NR	NR	NR	NR	Chronic rat & dog	↓ BW/FC; ↑ cholesterol; uterine histopath (dog); tremors (dog)	100	100	1.0
Emamectin	15-day mouse neurotox.	Tremors at day 3	0.075	100	0.00075	15-day mouse	Moribund sac; clin. neurotox; ↓ BW/FC; neuropathology	0.075	300	0.00025
Fipronil	Acute rat neurotox.	↓ hindleg splay	0.5	100	0.005	Chronic/onco rat	Neurotox clin obs. ; numerous clin. chem. changes; altered thyroid hormones (↑ TSH; ↓ T4)	0.019	100	0.00019
Chlorpyrifos	28-day human oral c	No plasma ChEI at 1 and 3 days postdose	0.1	10	0.01	28-day human oral c	Plasma ChEI; cholinergic clinical signs	0.03	10	0.003
CHEMICAL X	Develop. neurotox.	Maternal clinical observations	40	100	a	Dog subchronic	Hepatopathology	1.35	100	b

Table 9. Risk assessment profile: studies and endpoints selected for risk assessment of pesticides

Chemical	Acute					Chronic				
	Study	Endpoint	NOEL	UF	RfD	Study	Endpoint	NOEL	UF	RfD

NOEL and RfD are expressed as mg/kg/day. The uncertainty factors presented in this table do not include the FQPA Safety Factor.
NR = Not required (no appropriate study or endpoints available).
a = Non food-use chemical; Short term (1-7 days) dietary assessment for incidental hand-to-mouth oral residential exposure to children; MOE approach used (not RfD).
b = Non food-use chemical; Intermediate term (1 week -several months) dietary assessment for incidental hand-to-mouth oral residential exposure to children; MOE approach used (not RfD).
c = These endpoints are representative of the current RfD determinations and may be revisited pending the outcome of the December, 1998 SAP/SAB meeting on human testing.

Table 10. Comparison of NOELs from selected studies in rats and NOELs selected for dietary risk assessment

Chemical	Developmental Neurotoxicity		Developmental Rat		Reproduction		Neurotoxicity		Study Used in Risk Assessment	
	Maternal	Offspring	Maternal	Fetal	Parental	Offspring	Acute	Subchronic	Acute	Chronic
Aldicarb	0.05	0.05	0.125	0.125	0.4	0.7	<0.05	<0.05	0.01	0.01
Carbaryl	1.0	1.0	10	10	100	≥200	<10	1.0	1.0	1.4
Carbofuran	1.7	1.7	1	3	1	1	NS	<2.4	0.5	0.05
Molinate	6.9	<1.8	35	2.2	<0.4	0.4	<25	<4.0	<1.8	<0.3
DEET	90	90	250	250	<25	≥250	50	90	NR	100
Emamectin	0.6	0.1	2	4	0.6	0.6	<27.4	1.0	0.075	0.075
Fipronil	0.9	0.9 a	4	20	0.25	2.5	2.5	0.3	0.5	0.019
Chlorpyrifos	<0.3	1	0.1	≥15	0.1	1	10	≥15	0.1	0.03
CHEMICAL X	40	125	40	125	5.7	>197.9	NS	5.7	40 b	1.35 b
1,1,1-TCE c	750	>750	1000	3000	NR	NR	NR	630	ND	ND
TGME d	1650	300	1250	625	NR	NR	NR	400	ND	ND
Isopropanol d	700	>1200	400	400	100	100	4150	4150	ND	ND

NOELs expressed as mg/kg/day. When separate dose values were obtained for each generation, sex, etc., the lowest value was used in the table.
NS = Not submitted to the Agency; NR = Not required; ND = Not determined.
a = A separate developmental NOEL was established at 0.05 mg/kg/day, based on decreased pup body weight at 0.9 mg/kg/day.
b = Non-dietary, short- and intermediate-term residential oral exposure to children (hand-to-mouth).
c = NOAEL expressed as ppm; the developmental neurotoxicity study was a gavage study, and for purposes of comparison with all other studies which were dosed via inhalation, the oral doses used were converted to ppm.
d = NOAEL expressed as mg/kg/day; adult neurotoxicity studies were conducted by inhalation and the concentrations in ppm were converted to mg/kg/day for purposes of comparison.

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1,1,1-trichloroethane:

Triethylene glycol monomethyl ether:

Isopropanol:

<u>Multigeneration Reproduction Studies</u>:

Aldicarb: Lemen, J.K. (1991) Two-generation reproduction study in rats with aldicarb. Hazleton Washington, Inc., Vienna, VA, study No. 656-157, December, 17, 1991. MRID 42148401. Unpublished.

Carbaryl:

Carbofuran:

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DEET: Schardein, J.L. (1989) Evaluation of DEET in a two generation reproduction/fertility study in rats. International Research and Development Corp., Mattawan, MI, study No. 555-004, January 23, 1989. MRID 40979001. Unpublished.

Emamectin: Lankas, G.R. (1993) MK-0244: Two-generation dietary reproduction study in rats. Merck Research Laboratories, West Point, PA, study No. TT #91-715-0, May 12, 1993. MRID 42851511. Unpublished.

Fipronil: King, V.C. (1992) M&B 46030: reproductive performance study in rats treated continuously through two successive generation. Life Science Research Ltd., study No. LSR 92/RHA425/0309, June 26, 1992. MRID 42918647. Unpublished.

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CHEMICAL X: Hoberman, A.H.. (1993) Reproductive and neurobehavioral effects of sample No. 38674 administered orally via the diet to Crl:CD®BR VAF/Plus® Rats for two generations. Argus Research Laboratories Inc., Horsham, PA, laboratory study No, 91-02, December 17, 1993. MRID 43883913. Unpublished.

1,1,1-trichloroethane: Not conducted

Triethylene glycol monomethyl ether: Not conducted

Isopropanol:

<u>Acute Neurotoxicity Studies</u>:

Aldicarb: Robinson, K., W. Brooks, and B. Broxup. (1994) An acute study of the potential effects of orally administered aldicarb, technical grade on behavior and neuromorphology in rats, Bio-Research Laboratories, Ltd., Senneville, Quebec, study No. 97235, September 28, 1994. MRID 43442301. Unpublished.

Carbaryl: Brooks, W. et al. (1995) An acute study of potential effect of a single orally administered dose of carbaryl, technical grade, on behavior and neuromorphology in rats. Bio-Research Laboratories, Ltd., Senneville, Quebec, study No. 97389, 1995. MRID 43845201. Unpublished.

Carbofuran: Not submitted

Molinate: Horner, J.M. (1994) Molinate: acute neurotoxicity study in rats, Zeneca Central Toxicology Laboratory, Cheshire, UK, study No. AR5591, March 22, 1994. MRID 43188001. Unpublished.

DEET: Schardein, J.L. (1990) Neurotoxicity evaluation in rats following acute oral exposure to DEET. International Research and Development Corp., Mattawan, MI, study No. 555-017, January 23, 1990. MRID 41368501. Unpublished.

Emamectin: Manson, J. (1992) MK-0243. Acute oral neurotoxicity study in rats. Merck Research Laboratories, West Point, PA, study No. TT #89-069-0, December 21, 1992. MRID 42743618. Unpublished.

Manson, J. (1992) MK-0243. Acute oral neurotoxicity study in rats #2. Merck Research Laboratories, West Point, PA, study No. TT #89-0129-0, December 18, 1992. MRID 42743619. Unpublished.

Fipronil: Gill, M.W., C.L. Wagner, and D.D. Driscoll. (1993) M&B 46030: single exposure peroral (gavage) neurotoxicity study in Sprague-Dawley® rats. Bushy Run Research Centre, Export, PA, study No. 91N0099, April 26, 1993. MRID 42918635. Unpublished.

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CHEMICAL X: Not submitted

1,1,1-trichloroethane:

Triethylene glycol monomethyl ether: Not conducted

Isopropanol:

<u>Subchronic Neurotoxicity Studies</u>:

Aldicarb: Robinson, K., W. Brooks, and B. Broxup (1995) A 13-week study of the potential effects of orally administered aldicarb technical on behavior, neurochemistry and neuromorphology in rats, Bio-Research Laboratories, Ltd., Senneville, Quebec, study No. 97234, October 4, 1995. MRID 43829602. Unpublished.

Carbaryl: Robinson, K. And B. Broxup (1996) A 13-week study of the potential effects of orally administered carbaryl, technical grade, on behavior, neurochemistry and neuromorphology in rats. Bio-Research Laboratories, Ltd., Senneville, Quebec, laboratory project no. 97390, September 24, 1996. MRID 44122601. Unpublished.

Carbofuran: Freeman, C. (1994) Carbofuran technical: subchronic neurotoxicity study in rats. FMC Corporation Toxicology Laboratories, study No. A92-3705, February 25, 1994. MRID 43163401. Unpublished.

Molinate: Horner, J.M. (1994) Molinate: subchronic neurotoxicity study in rats. Zeneca Central Toxicology Laboratory, Cheshire, UK, study No. PR0949, May 10, 1994. MRID 43270701.

Unpublished.

DEET: Schardein, J.L. (1990) Neurotoxicity evaluation in rats following multigeneration exposure to DEET. International Research and Development Corp., Mattawan, MI, study No. 555-015, January 23, 1990. MRID 41368401. Unpublished.

Emamectin: Gerson, R.L. (1992) MK0244. Fourteen-week dietary neurotoxicity study in rats. Merck Research Laboratories, West Point, PA, study No. TT #91-006-0, December 18, 1992. MRID 42743628. Unpublished.

Fipronil: Driscoll, C.D. and J.M. Hurley (1993) M&B 46030: ninety-day dietary neurotoxicity study in Sprague-Dawley rats. Bushy Run Research Center, Export, PA, study No. 92N1074, September 15, 1993. MRID 43291703. Unpublished.

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<u>RfD, Hazard ID, and/or Toxicology Endpoint Selection Committee Reports</u>:

Aldicarb: Sette, W.F., Aldicarb: report of the Hazard Identification Assessment Review Committee (meeting of July 23, 1998, September 15, 1998.

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Molinate:Taylor, LA. And J. Rowland, Molinate: report of the Hazard Identification Assessment Review Committee (meeting of November 16, 1998), in draft.

DEET: Phang, W., DEET: toxicology endpoint selection document (meeting of October 18, 1995), January 4, 1996.

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Rowland, J., Fipronil reevaluation: report of the Hazard Identification Assessment Review Committee (meeting of April 22, 1998), May 7, 1998.

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Appendix A-1
Triggers for recommending DNT studies

Recommendations regarding procedures designed to trigger the need for conducting a developmental neurotoxicity study had been proposed by the Agency (Timm, 1987 and Rees, 1988), and by the 1989 workshop (Levine and Butcher, 1990) that was held to discuss the guideline. The approaches included the presumption that the developmental neurotoxicity study would be conducted as a second tier evaluation, and that the need for a developmental neurotoxicity study would be based on a weight-of-the-evidence review of all available data for each chemical, including the prenatal developmental toxicity studies and multigeneration reproduction study. The criteria generally used in the determination were reviewed and approved by a Scientific Advisory Panel in 1987 and were reconfirmed by a 1995 SAP. They specified that developmental neurotoxicity testing should be:

a) mandatory if the substance has been shown to cause CNS malformations;
b) strongly considered if the substance has been shown to cause neuropathology/neurotoxicity in adults or affect brain weight in weanlings;
c) strongly considered if the substance is a hormonally-active compound (pituitary, thyroid, sex hormones), or affects sexual maturation;
d) considered if the substance causes other types of developmental toxicity.

Additionally, since the developmental neurotoxicity study has not yet been included in 40 CFR Part 158, the Office of Management and Budget (OMB) has specified (OMB No. 2070-0107, 5/8/91) that larger-scale Data-Call-Ins (DCIs) can be issued for developmental neurotoxicity studies only if certain criteria are met. These criteria were that:

a) neurotoxicity is observed in developing or adult animals following exposure to the compound;
b) behavioral/functional changes are produced by direct effect of the compound on the nervous system;
c) the compound acts to significantly modify hormonal responses associated with the development of the nervous system leading to significant developmental effects; or
d) the compound exhibits a strong structure-activity relationship to a known neurotoxicant

In response to concerns regarding the completeness of data available for assessing hazard to infants and children, in a March, 1998 presentation to the Scientific Advisory Panel, the Health Effects Division (HED) of OPP proposed a further elaboration on the scientific rationale used to determine the need for a developmental neurotoxicity study. This decision logic has been used in HED from that time forward.

The requirement of the developmental neurotoxicity testing for pesticides is based on whether the chemical profile meets one or more of the following criteria.

The substance has been shown to:

1. cause CNS malformations following prenatal exposure;
2. affect brain weight in offspring, which does not appear to be related solely to general growth retardation, following pre- and/or postnatal exposure;
3. cause neuropathology in developing or adult animals or neuropathy in humans;
4. cause persistent functional changes in the offspring which may be the result of effects on the nervous system;

5. act to significantly modify hormonal responses associated with the development of the nervous system, leading to significant developmental effects (e.g., effects on sexual maturation).

In addition, a weight-of-evidence assessment of the data base is conducted, and all information pertinent to the assessment of neurotoxic potential of the chemical is considered when determining the need for a developmental neurotoxicity study. This could include factors such as:

a) acute behavioral/functional changes are produced in adult animals by an effect of the compound on the nervous system;
b) the compound exhibits a structure-activity relationship to a known neurotoxicant or neuroactive chemical;
c) evidence of developmental toxicity to fetal tissues, organs, and/or systems (other than the CNS) generates concern regarding potential effects on functional development of affected fetuses; or
d) the potency of the chemical, the persistence of neurotoxic effects, or the partitioning of effects in the animal model (e.g., brain cholinesterase inhibition that occurs at a much lower dose than elicits plasma cholinesterase inhibition) generates an additional level of concern.

Even in the absence of one or more of the specific criteria listed in items 1-5 above that would trigger the need for a developmental neurotoxicity study, the weight-of-evidence assessment could provide sufficient concern to result in this conclusion.

At the Scientific Advisory Panel meeting held in March, 1998, the Panel recommended that the Agency consider including two additional criteria to trigger the developmental neurotoxicity study: if the chemical is 1) neurotoxic to insects or 2) causes deficits in learning and memory or other cognitive effects (in mammals). OPP has not yet implemented these criteria, since the question of when to conduct developmental neurotoxicity studies is currently being considered by the 10X Task Force, an interoffice Agency workgroup that was formed to come to agreement on various issues surrounding the application of the 10X FQPA safety factor.

Appendix A-2. Criteria used to determine the need for a developmental neurotoxicity study (chemicals for which a DNT study has been recommended but not yet received/reviewed by OPPTS)

Chemical	Chemical Class	Criteria					Weight-of-Evidence Considerations				Notes
		1: Malf	2:BrWt	3:NPath	4:Func	5:Horm	a:Behav	b:SAR	c:Dev	d:Poten	
1	Organophosphate			X			X	X		X	3: Literature studies
2	Organophosphate			X			X	X		X	d: Potency
3	Organophosphate			X			X	X			
4	Organophosphate			X			X	X			3: Literature studies
5	Methyl dithio-carbamate	X					X	X			a: Motor activity changes over wide range of doses; no ChEI
6	Thiocarbamate	X		X			X	X			
7	Dimethyl dithio-carbamate	X				X	X				
8	Pyrethroid			X			X	X			
9	Organochlorine						X	X			
10	Organochlorine					X	X	X			
11	Chloronicotine						X	X			
12	Phenylpyrrole			X							
13	Azole						X	X	X		c: Cleft palate and ↑ 2nd generation fertility
14	Azole						X	X			b: Known mechanism of action
15	Azole	X						X			
16	Benzamidazole	X						X			

Appendix A-2. Criteria used to determine the need for a developmental neurotoxicity study (chemicals for which a DNT study has been recommended but not yet received/reviewed by OPPTS)

Chemical	Chemical Class	Criteria					Weight-of-Evidence Considerations				Notes
		1: Malf	2:BrWt	3:NPath	4:Func	5:Horm	a:Behav	b:SAR	c:Dev	d:Poten	
17	Benzoylisoxazole			X			X				
18	Benzoic acid			X			X				
19	N-trihalomethylthio	X									
20	Phosphonoamino acid			X			X				
21	Sulfonylurea	X								X	d: ↓ Brain weight in adults
22	Acetamide			X			X				
23	Acetamide			X		X	X				
24	Formamidine						X	X			Central stimulant
25	Phosphonium sulfate			X			X				
26	Thiocarbamate			X				X			
27	Chlorinated hydrocarbon					X	X	X			5: Literature studies revealed neuroendocrine alterations in fetal development (rat)

Key to Criteria:
1 = CNS malformations
2 = Brain weight in offspring
3 = Neuropathology
4 = Persistent functional changes in offspring
5 = Hormonal modification

Key to Weight-of-Evidence Considerations:
a = Non-persistent behavioral/functional changes in adults
b = SAR to known neurotoxic/neuroactive chemical
c = Other developmental toxicity that could affect functional development
d = Potency, persistence of neurotoxicity, partitioning of effects